RBP4 (mouse/rat) ELISA Kit

(Catalog #K4913-100; 100 assays; Store kit at 4°C)

I. Description:

Retinol binding protein (RBP) 4 is the only specific transport protein for vitamin A in the circulation whose function is to deliver vitamin to target tissues. In obesity and type 2 diabetes, expression of Glut4 is significantly impaired in adipocytes. Glucose transport via Glut4 is the rate-limiting step for glucose use by muscle and adipose tissue. Adipocyte-specific deletion of Gluts leads to notable elevation of mouse RBP4 causing systemic insulin resistance, and that reduction of RBP4 improves insulin resistance. This identified a novel role of RBP4 in regulating insulin action and RBP4 is recorded as an adipocyte-derived hormone. The RBP4 (mouse/rat) ELISA Kit is to be used for the *in vitro* quantitative determination of mouse or rat RBP4 in serum, urine and cell culture supernatant. This assay is a sandwich ELISA which utilizies a 96-well microtiter plate which was pre-coated with a monoclonal antibody and a purified polyclonal detection antibody. A HRP-conjugated anti-IgG, peroxidase and TMB (3,3',5,5'-tetramethylbenzidine) is added to generate a color intensity directly proportional to the concentration of RBP4 in the samples. This ELISA is specific for the measurement of natural and recombinant mouse and rat RBP4. It does not cross-react with human RBP4, mouse adiponectin, mouse resistin, mouse Nampt, rat adiponectin, rat resistin, rat Nampt, mouse RELM-α, mouse RELM-β, human leptin, rat RELM-α, mouse 41BBL, mouse CD137, mouse Tim3, human TNF-α. The assay range is 0.188 – 12 ng RBP4/ml and a detection limit of 60 pg/ml (based on adding two standard deviations to the mean value of the (50) zero standards).

II. Kit Contents:

Component	100 Assays	Part Number
Pre-coated Microtiter Plate	1 ea (12 x 8 well strips	K4913-100-1
Wash Buffer (10X)	50 ml	K4913-100-2
Diluent (5X)	50 ml	K4913-100-3
Detection Antibody	12 ml	K4913-100-4
Detector 100X (Hrp conjugated anti-IgG)	150 µl	K4913-100-5
Mouse RBP4 Standard (lyophilized,24 ng)	1 vial	K4913-100-6
Mouse RBP4 QC Sample (lyophilized)	1 vial	K4913-100-7
Substrate Solution I (TMB)	6 ml	K4913-100-8
Substrate Solution II (Peroxidase)	6 ml	K4913-100-9
Stop Solution	12 ml	K4913-100-10
Plate Sealers	3 each	K4913-100-11

III. Storage Conditions:

Reagents must be stored at 2 - 8°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

IV. Assay Procedure (Read the ENTIRE Protocol Before Proceeding)

1. Test Samples/Standards/QC Sample: (We recommend these be run in duplicate)

a) **Serum**: Use a serum separator tube. Let samples clot at room temperature for 30 min before centrifugation for 20 min at 1000 x g. Assay freshly prepared serum or store serum in aliquots at -20°C for future use. Avoid repeated freeze/thaw cycles. Serum should be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use. As a starting point, 1/10,000 dilution is suggested. If samples fall outside the assay range a lower or higher dilution may be required.

b) **Urine:** Aseptically collect the urine, voided directly into a sterile container. Assay immediately or aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Urine should be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use. As a starting point, 1/10 dilution is suggested. If samples fall outside the assay range a lower or higher dilution may be required.
Note: **Serum, Plasma, Urine** or **Cell Culture Supernatant** have to be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use.

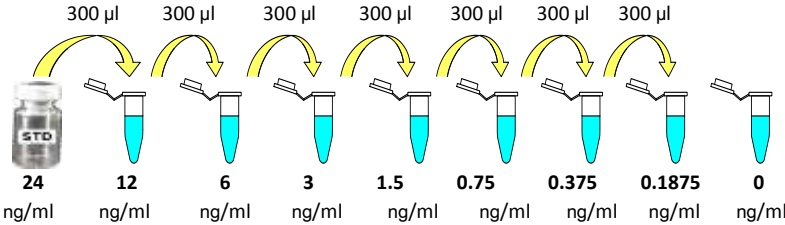
c) **QC Sample**: Reconstitute Mouse RBP4 QC Sample with 1 ml of dH_2O. Mix the QC Sample to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The QC Sample is ready to use-do not dilute it (refer to the C of A for current QC Sample concentration).

d) **Standards**: Reconstitute Mouse RBP4 Standard with 1 ml of dH_2O to produce a stock solution (24 ng/ml). Mix the Stock solution to ensure complete reconstitution. Allow to sit for a minimum of 15 min. The reconstituted standard should be aliquoted and stored at -20°C.

e) Prepare 1X Diluent: Dilute 5X Diluent 1:4 with dH_2O.

f) Prepare Standard Curve using 2-fold serial dilutions with 1X Diluent:

To obtain	Add	Into
12 ng/ml	300 µl of RBP4 (24 ng/ml)	300 µl of 1X Diluent
6 ng/ml	300 µl of RBP4 (12 ng/ml)	300 µl of 1X Diluent
3 ng/ml	300 µl of RBP4 (6 ng/ml)	300 µl of 1X Diluent
1.5 ng/ml	300 µl of RBP4 (3 ng/ml)	300 µl of 1X Diluent
0.75 ng/ml	300 µl of RBP4 (1.5 ng/ml)	300 µl of 1X Diluent
0.375 ng/ml	300 µl of RBP4 (0.75 ng/ml)	300 µl of 1X Diluent
0.1875ng/ml	300 µl of RBP4 (0.375 ng/ml)	300 µl of 1X Diluent
0 ng/ml	300 µl of 1X Diluent	Empty tube



2. Reagent Preparation: (Prepare just the appropriate amounts for the assay)

a) **1X Wash Buffer:** Dilute 10X Wash Buffer 1: 9 with dH_2O to obtain 1X Wash Buffer.

b) **1X Diluent:** Dilute 5X Wash Buffer 1: 4 with dH_2O to obtain 1X Diluent.

c) **1X Detector**: Dilute 100X Detector 1: 99 with 1X Diluent to obtain 1X Detector.

d) **Substrate Solution**: Equal volumes of Substrate Solutions I and II must be mixed together.

Note: The diluted Detector must be used within 1 hr of preparation; Substrates I and II should be mixed within 15 min of use (Protect Substrate Solution from light)

3. Assay Protocol:

a) Determine the number of 8-well strips needed for assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.

b) Add 100 µl of the Standards, Samples and QC Sample into the appropriate wells in duplicate.

c) Cover plate with plate sealer and incubate for 1 hr at 37°C.

d) Aspirate and wash x 3 with 300 µl of 1X Wash Buffer.

e) Warm Detection Antibody to room temperature. Add 100 µl to each well and tap gently on the side of the plate to mix.

f) Cover plate with plate sealer and incubate for 1 hr at 37°C.

g) Aspirate and wash x 3 with 300 µl of 1X Wash Buffer.

h) Add 100 µl of the 1X Detector to each well.

i) Cover plate with plate sealer and incubate for 1 hr at 37°C.

j) Remove plate from 37°C, aspirate and wash x 5 with 300 µl of 1X Wash Buffer.

k) After last wash, tap inverted plate on a stack of paper towels. Complete removal of liquid is essential for good performance.

l) Add 100 µl of the mixed Substrate Solution to each well.

m) Allow the color to develop at room temperature in the dark for 20 min.

n) Stop the reaction by adding 100 µl of Stop Solution to each well.

o) Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
Caution: Stop Solution is a Corrosive Solution

p) Measure the OD at 450 nm in an ELISA plate reader within 30 min.

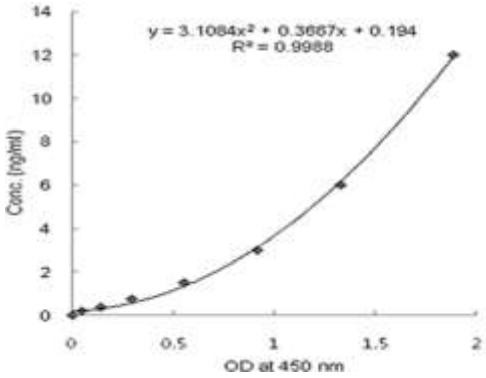
3. Calculations:

a) Average the duplicate readings for each Standard, QC Sample and Test Sample and subtract the average blank value (obtained with the 0 ng/ml point).

b) Generate a Standard Curve by plotting the average absorbance on the horizontal (X) axis vs. the corresponding concentration (µg /ml) on the vertical (Y) axis. (See Typical Data below)

c) Calculate the Test Sample RBP4 concentrations by interpolation of the Standard Curve regression curve as shown below in the form of a quadratic equation.

d) If the Test Samples were diluted, multiply the interpolated values by the dilution factor to calculate the corrected mouse or rat RBP4 concentrations.



VI. Performance Characteristics:

1. Intra-assay Precision: (6) samples of known concentration were assayed in replicates (8) times to test precision within an assay.

Samples	Mean (µg/ml)	SD	CV (%)	n
1	14.23	0.88	6.20	8
2	24.04	0.28	1.18	8
3	4.88	0.13	2.57	8
4	15.90	0.53	3.35	8
5	28.50	0.98	3.44	8
6	9.25	0.44	4.79	8

2. Inter-assay Precision: (6) samples of known concentration were assayed in (8) separate assays to test precision between assays.

Samples	Mean (µg/ml)	SD	CV (%)	n
1	15.09	0.80	5.31	8
2	23.16	1.21	5.21	8
3	5.18	0.14	2.78	8
4	16.77	1.17	6.95	8
5	28.94	1.89	6.52	8
6	9.60	0.48	5.04	8

3. Recovery: Mouse and rat serum samples were spiked with known concentrations of mouse and rat RBP4. The recovery averages were 98 % (range from 88 % to 100 %)

Samples	Average Recovery (%)	Range (%)
1	101.87	94-108
2	97.57	90-104
3	100.45	94-106
4	92.94	88-98

4. Expected Values: RBP4 serum levels range in mouse or rat samples from 5 to > 40 µg/ml. RBP4 urine levels range in mouse or rat samples from 5 to > 100 ng/ml.

Technical Hints and Limitations:

- It is recommended that all standards, QC sample and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep Substrate Solution protected from light.
- The Stop Solution consists of phosphoric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

Troubleshooting:

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

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